Laboratory Support for Influenza Vaccine Development – Potency Reagents

HHS International Vaccine
Infrastructure Workshop
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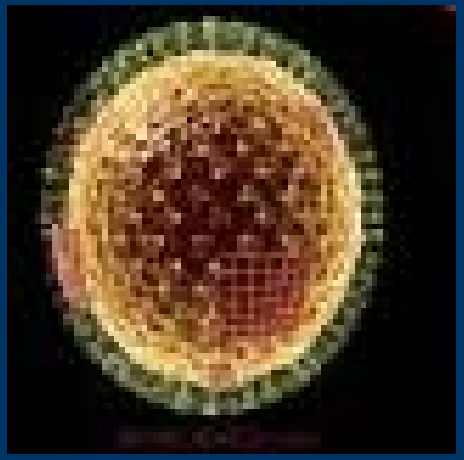

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Current Influenza Vaccines in the U.S.

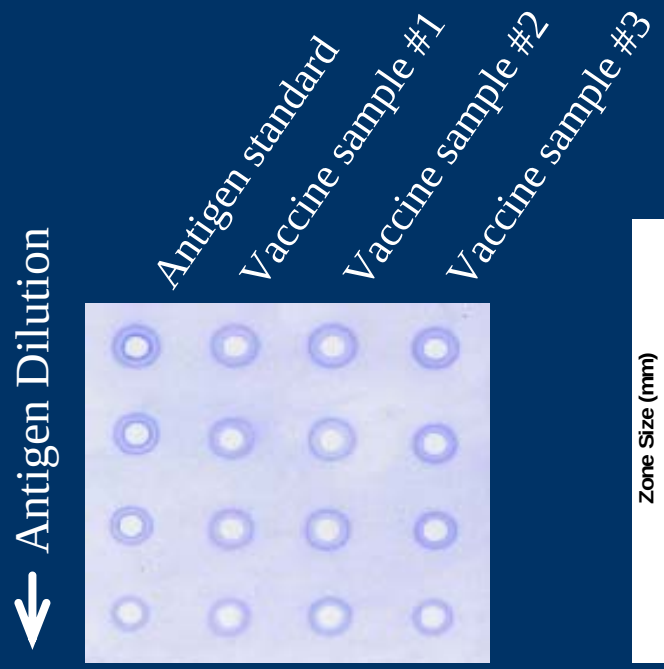
- Majority of current influenza vaccines (seasonal and pandemic) are inactivated/split vaccines manufactured in embryonated eggs
  - Fluzone (Sanofi-Pasteur)
  - Fluvirin (Novartis)
  - Fluarix (GSK)
  - FluLaval (ID Biomedical [GSK])
  - Afluria (CSL)
  - Agriflu (Novartis)
- 1 manufacturer of live attenuated influenza vaccine
  - FluMist (MedImmune)

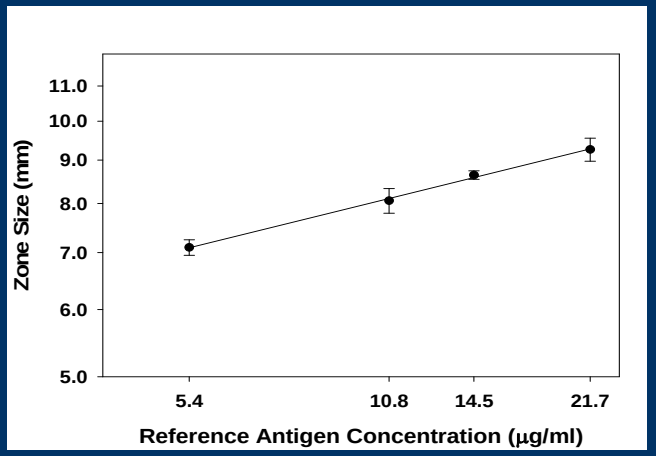
Influenza Vaccine Potency

- Potency of live attenuated influenza vaccines determined by measuring virus titer (focus-forming assay)
  - No standard involved
- Potency of inactivated influenza vaccines determined by single radial immunodiffusion assay (SRID)
  - Assay dependent on availability of standards to measure antigen content (HA) in vaccines
  - Standards specific for each virus strain
  - Standards preparation & calibration is a routine aspect of influenza vaccine manufacture and production
  - Current process of standards preparation & calibration takes weeks/months
  - Standards necessary to ensure that multiple inactivated vaccine products produce the same clinical benefit

SRID Potency Assay

- Assay based on diffusion of virus antigen (e.g., detergent-disrupted virus or vaccine) through agarose gel containing HA-specific antibody
- Diameter of the precipitin ring proportional to the antigen concentration
- Standard curve used to quantify HA in vaccine samples





SRID Potency Assay

- Assay strengths:
  - Not technically demanding
  - Inter-laboratory reproducibility
  - Measures an antigenic form of HA
  - Measured HA content correlates with clinical benefit
- Assay weaknesses:
  - Reagent standardization is critical and time-consuming
  - Dependent upon strain-specific reagents
  - Limited sensitivity (i.e., range)
  - Large quantities of reagents needed to support world-wide vaccine manufacture

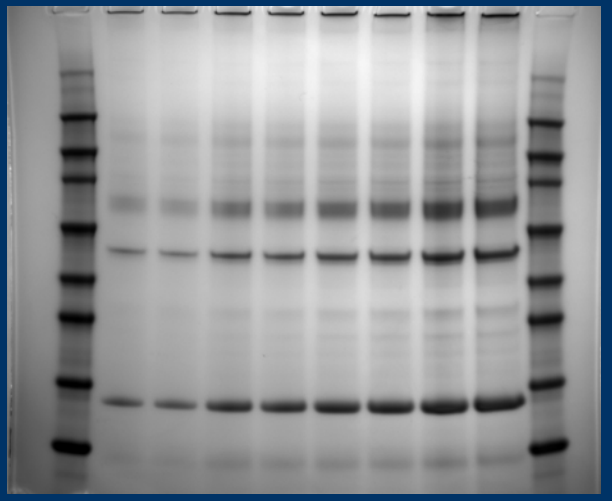
Preparation and Calibration of Potency Reagents

- A specific antigen standard and antibody standard is required for each vaccine virus strain
  - Lyophilized and supplied together
  - Reference antigen has an HA value assigned
  - Working dilution provided for reference antibody
- Antigen standard is whole-inactivated virus
  - Primary standard prepared for calibration process
  - Secondary (lyophilized) standard supplied for potency determination
- Antibody standard is hyper-immune sheep serum
- Preparation of antigen and antibody standards proceeds in parallel
  - Distributed by WHO Essential Regulatory Laboratories (CBER/FDA; NIBSC; NIID; TGA)

Calibration of Antigen Reagents

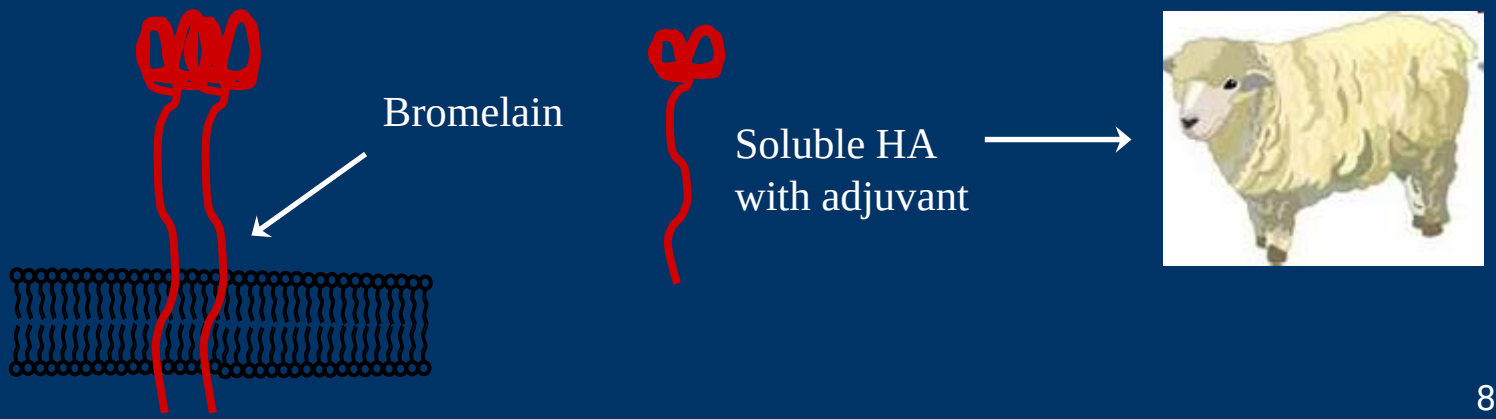
- The HA content of a primary standard (inactivated virus - liquid) is determined by physical-chemical methods
  - Total protein - nitrogen analysis or Lowry
  - % HA – SDS/PAGE & densitometry
  - HA value assigned from independent analyses of the WHO ERLs
- Reference antigen prepared at industrial scale, aliquoted and lyophilized
- HA content of the reference antigen assigned by SRID using primary standard and strain-specific antibody
  - Independent analysis by WHO ERLs
- Reagents distributed as soon as available
- Entire process takes several weeks

HA quantification of primary standard by protein determination & gels/densitometry - DPQ/CBER



Preparation of Strain-Specific Antibody Reagents

- Virus is digested with bromelain to remove HA from virus particles
- HA, missing transmembrane portion, is purified
- Animals immunized multiple times to generate hyper-immune antiserum
- Antiserum tested for suitability in SRID
- Reference antiserum aliquoted and lyophilized
- Distributed along with reference antigen



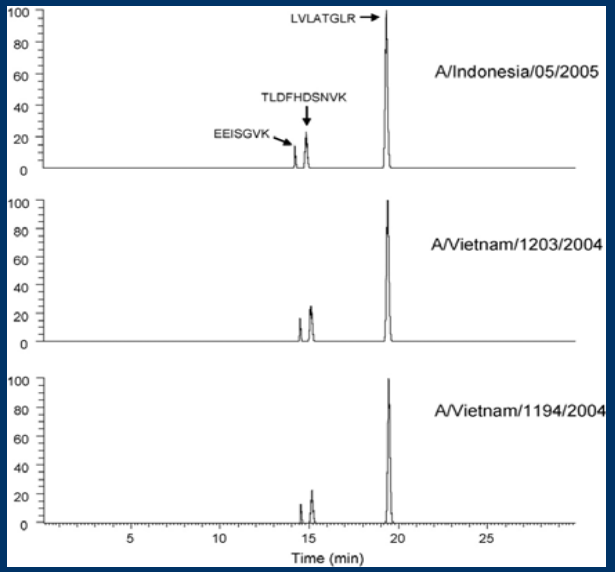
Current and Future Challenges

- Improving the current process of reagent preparation and calibration
  - Expediting calibration process
  - Developing back-up procedures to ensure reagent availability
- Developing new methods for potency determination
  - Increasing sensitivity
  - Methods that are not antibody dependent
- Developing new technologies to determine potency of new-generation vaccines
  - Cell culture – derived vaccines
  - Adjuvanted vaccines
  - DNA vaccines
  - Recombinant protein based vaccines including virus-like particles

Improving the Current Reagent Calibration Process

- Recently reported MS technique (CDC) uses isotope dilution approach to quantify HA in complex mixtures (Williams et al, CDC)
- Multi-Center collaboration (FDA/CDC) set-up to evaluate use of MS to determine absolute quantity of HA in primary influenza standard
- If correlated with current methods, time necessary for calibration could be reduced from weeks to days

HA quantification by MS analysis



Developing Alternatives for Reagent Production

- Current techniques require availability and preparation of virus, and HA purification before immunization to generate strain-specific antibody - not always successful (e.g., H5N1, pandemic H1N1)
- Using recombinant techniques, potency antibody can be generated in absence of virus and not dependent upon HA purification

HA content (µg/ml) in vaccine lots determined by SRID using traditional and alternative potency antibody

	HA (µg/ml)					
Potency Antibody[1]	A/Vietnam (traditional)	A/Vietnam (alternative)	A/Indonesia (traditional)	A/Indonesia (alternative)	A/California (traditional)	A/California (alternative)
Vaccine						
A/Vietnam/1203/2004 (H5N1)						
Lot 1[3]	**116±11**	**100±11**	ND[2]	ND	ND	ND
Lot 2	**116±20**	**112±30**	124±21	164±4	ND	ND
A/Indonesia/5/2005 (H5N1)						
Lot 1	ND	ND	**185±18**	**303±24**	ND	ND
Lot 2	664±86	716±68	**664±87**	**646±50**	ND	ND
A/California/7/2009 (H1N1)						
Lot 1	ND	ND	ND	ND	**79±9**	**72±2**
Lot 2	ND	ND	ND	ND	**925±137**	**1017±34**
Lot 3	ND	ND	ND	ND	**70±8**	**70±9**

New Methods for Potency Determination

- Various techniques being explored as alternatives to SRID potency assay including:
  - ELISA
  - Mass spectrometry
  - HPLC
- Used in 2009 pandemic for formulation of clinical trial material (HPLC)
  - Not yet shown to measure an antigenic form of HA that can be correlated with clinical benefit
  - Standards may still be needed but not antibody for some methods

Summary

- Influenza vaccines present unique considerations for product development including the need to standardize vaccines from multiple manufacturers
- Potency reagent development and calibration is time consuming and always a potential bottleneck in production of inactivated influenza vaccines
- Improvements are needed in the current process to ensure timely availability of sufficient reagents
- Development of new techniques and methods for potency evaluation and standards preparation are needed for new-generation influenza vaccines, including some on the short-term horizon